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Growth performance of *Vigna radiata* (L.) R. Wilczek in petrol contaminated soil amended with *Centrosema pubescens* benth

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ABSTRACT

This study was carried out at the University of Port Harcourt, Green House to investigate the growth performance of *Vigna radiata* in petrol contaminated soil amended with *Centrosema pubescens*. The contamination levels of 2%, 4% and 8% v/w were used to contaminate 20kg of loamy soil amended with 5kg of *Centrosema pubescens* at various levels of petrol contaminations, alongside a control. The growth parameters (plant height, leaf area, and number of leaves) and plant biomass (fresh weight and dry weight) were examined and the data was subjected to analysis of variance (ANOVA). Based on the result, the growth parameters and plant biomass reduced with increased levels of contaminations, however control significantly ($P<0.05$) had greatest growth performance in

all growth parameters and plant biomass among all the treatments studied. This showed the potential of *Centrosema pubescens* in improving the soil component and reduction in the level of toxicity of petrol contaminated soil and thus suggests that *Centrosema pubescens* is an effective organic supplement for the remediation of petrol contaminated soil where *Vigna radiata* is cultivated.

Keywords: *Vigna radiata, Centrosema pubescens,* growth parameters, petrol

1. INTRODUCTION

The word “pollution” can be referred to as an undesirable state of the natural environment being contaminated with harmful substances as a consequence of human activities. Pollution is often classed as point source or non-point source. Substances that cause adverse change in the environment are known as pollutants (Wilson, 2005).

Contamination is an undesirable change in the physical, chemical or biological characteristics of the air, water or soil that can harmfully affect the health, survival or activities of humans or their living organism (Nyananyo, 2008). There have been several methods of remediating Premium Motor Spirit (PMS) from the soil which includes phytoremediation; describes as the direct use of living green plants for in situ, or in place, removal, degradation, or containment of contaminants in soils, sludges, sediments, surface water and groundwater. Bioaugumentation is the addition of organisms to effect remediation of the environment. Biostimulation is the addition of organic or inorganic compounds to cause indigenous organisms to effect remediation of the environment.

Phytoremediation of petroleum polluted soil is one of the promising environmental and cost effective approaches to polluted area (Tanee and Kinako, 2008). The word petroleum (literally “rock oil” from the Latin Petra, “rock” or “stone”, and oleum, “oil”. Petroleum is a naturally occurring, yellow to black liquid found in geological formations beneath the earth’s surface. It is a complex mixture of hydrocarbon with small amount of organic compounds containing metallic constituents, particularly nickel, iron, copper and vanadium. The major sources of petroleum pollution activities are; deblasting activities of tankers, transportation operations, offshore and onshore exploration and production etc. Petrol has some physical characteristics which include density, stability and energy content. The term PMS (Premium Motor Spirit) is used most widely in Nigeria, where the largest petroleum companies call their product "premium motor spirit". Although "petrol" has made it in roads into Nigerian English, "premium motor spirit" remains the formal name that is used in scientific publications, government reports, and newspapers (Clark, 2002).

Vigna radiata is commonly known as Mung beans and alternatively known as Green gram. *Vigna radiata* is one of the many species that was recently moved from the genus Phaseolus to Vigna. The Mung bean was domesticated in Persia (Iran), where its progenitor (*Vigna radiata* subspecies sublubata) occurs wild. It is a plant species in the legume family. The Mung bean is a fast growing, warm season legume; it gets to maturity quickly where optimal temperature is about 28 – 30^{0}c. It is annual, erect or semi – erect, reaching a height of 0.15m – 1.25m. It is slightly hairy with a well developed root system with yellow flowers and fuzzy brown pods. The stems are many branched, sometimes twining at the tips; the leaves are alternate, trifoliate with elliptical to ovate leaflets, 5 – 18cm long and 3 – 15cm broad. The flowers (4 – 30cm) are papillonaceous, pale yellow or greenish in colour. The pods are long, cylindrical, hairy and pending. They contain 7 – 20 small, cube shaped seeds. The seeds are variable in colour. They are usually green but can also be yellow, olive brown. The seeds are prolific and ripen uniformly (Fuller, 2006). It is used as a cover crop before or after cereal crops. It makes good green manure. It fixes nitrogen to the soil that can provide large amounts of biomass and nitrogen to the soil. It helps as a soil improver. Mung beans is generally eaten either whole (with or without skins) or as bean sprouts. The starch is also extracted from them to make jellies (Poehlman, 1991). They can be used in soups and casseroles. The crop is grown for hay, bean sprouts is eaten as vegetable. It has the potential for contributing to both proteins and calorie intake of humans and animals. It is a high source of nutrients. It’s essential for vitamins A, C and E. It contains antioxidants. It helps to lower high blood pressure. It helps lower high cholesterol levels. It is very easy to digest. It also provides a high source of protein (Fuller, 2006).

Centrosema pubescens is a legume in the family Fabaceae. It is commonly known as Centro or butterfly pea. It is native to Central and South America and cultivated in other tropical areas as forage for livestock (Hutchinson et al., 1958). *Centrosema pubescens* is an herbaceous, climbing, perennial herb that can reach a height of 45cm. It can be cultivated in regions with rainfall ranging from 1000mm – 1750mm per year. The root system can reach up to 30cm in depth, usually in association with Rhizobium, nitrogen fixing bacteria. It is drought tolerance due to its deep root system. The vigorous stems twine into other plants for support. Stems grow and branch rapidly, producing a dense mass of branches and leaves on the soil. Leaves are trifoliate with elliptical leaflets, dark-green and glabrous above but whitish and densely tomentose below. Flowers are generally pale violet with darker violet veins, born in auxiliary racemes. Fruits are flat, long, dark-brown pod, containing up to 20 seeds. Seeds are spherical, dark-brown when ripe. It is propagated by seed, planted directly in the ground or broadcasted over a field. It can grow in any type of soil; from sandy soil to clay soil. Centro grows best in soil pH between 4.9 – 5.5. It can grow between 4 – 8 months. They can be found in waste places, on

river banks and on coconut plantation. It is used as forage and a source of protein to grazing cattle. It is grown as a cover crop because it naturally suppresses weeds and is very tolerant to drought. It is unable to tolerate cold temperatures but has very low soil and rainfall requirements. It is not suitable for human consumption but provides benefits through soil fertility and animal health. It can be intercropped with grasses, thus increasing the protein content of the cattle diet. The leaves can be used as a cheap source of protein for broiler chickens. It is a good source of calcium and potassium for animals (Nworgu, 2013).

2. MATERIALS AND METHODS

The study was carried out at the Centre for Ecological Studies, University of Port Harcourt. Soil samples were collected from the front of the agricultural farm, Abuja campus, University of Port Harcourt and were taken for analysis; Polythene bags were bought from the market and were perforated for easy drainage of water and aeration. Petrol were collected from AP filling station, seeds of *Vigna radiata* were sourced from Agricultural Development Programme (ADP), Rumuodomaya, Obio-Akpor in Rivers State. *Centrosema pubescens* were sourced from the back of petroleum engineering building, Abuja campus, University of Port Harcourt.

The experiment was laid out in completely randomized design with 5 treatments and 5 replicates giving a total of 25 bags. The seeds were planted randomly into the polythene bags. The amendments used were dried leaves of *Centrosema pubescens*. The different levels of pollution were (2%, 4%, and 8%) and the control (0%) receiving only water for the growth of the plant. The means of the various treatments were used in analyzing the data obtained. Each soil used for the experiment weighed 20kg.

Determination of seed viability was carried out to determine the degree of viability of seeds. Twenty five (25) seeds of *Vigna radiata* were plated in five (5) sterile Petri dishes. Five (5) seeds per plate.After soaking the seeds in water for some seconds prior to sowing the seeds. The Petri dishes were lined with filter paper moistened in water. After four (4) days the number of seeds that germinated was recorded by observing the protrusion of radicle and plumule. The *Vigna radiata* subjected to germination trial showed 80% germination and were considered fit for the experiment since the criteria for a seed germination test is from 60% germination.

Pollution treatment was carried out by adding different levels of petrol (PMS); 100ml, 200ml and 400ml to a measured quantity of soil (20kg). This was mixed thoroughly with water in the perforated polythene bags. The polythene bags were perforated in order to allow drainage of water and aeration. The contaminated soil samples were left to precondition for two (2) weeks for the petrol to acclimatize well to the soil before remediation is carried out and afterwards planting is done.

Centrosema pubescens was used to remediate the contaminated soil polluted with petrol (PMS). The *Centrosema pubescens* was collected fresh and kept in the screen house for it to be dried properly. After a period of 5 days the remediation treatment was carried out. The leaf litters were weighed (5kg) and added to the different levels of contamination (2%, 4% and 8%) in 20kg of soil and thoroughly mixed together and was allowed to precondition for 2 weeks for the amendment to be effective on the soil.

Vigna radiata (mung bean) were planted in perforated polythene bags at different treatment levels 0%, 2%, 4% and 8% v/w and watering was done immediately and subsequently every two days. Weeding was done by hand picking throughout the experiment. Five (5) seeds of *Vigna radiata* per bag were planted at 2cm sowing depth in the soil samples treated with different treatment levels. Seed germinated within four to six days. Seedlings were thinned down to two (2) per bag after two (2) weeks. Different analysis where carried out on the soil such as microbiological analysis, heavy metal analysis and physiochemical properties of experimental soil were also determined. The following parameters were analyzed: number of leaves, plant height, number of pods, and assessment of seed yield, fresh weight and dry weight. The leaves of the plant of the various treatments were counted visually. The number obtained was then recorded appropriately against each treatment. The plant height was measured with a meter tape in centimeters from the soil surface to the plant apex. The pods of each mung bean were counted visually. The pods are ready for harvest when the green colour changes to black. Matured pods were harvested as the study was being carried out and then stored in the screen house. The fresh weight was ascertained by weighing the mung bean plant immediately after harvest to avoid water loss. The fresh weight of the plant was ascertained by weighing on a digital weighing balance. The plant materials were dried in the screen house for 5 days. The dried plant materials were then collected from the screen house and weighed using an electronic digital weighing balance and the weights were recorded appropriately.

Data collected for each parameter were subjected to analysis of variance (ANOVA) using Microsoft Excel 2007 version. Means were compared using the least significant Difference (LSD).

3. RESULTS AND DISCUSSION

From table 1 below, T2 (2%) had the highest value for pH (5.02) from the soil samples analyzed, followed by T3(4%), T1(Control + amendment) and T4(8%). T0 (Control) had the lowest value for pH value (4.40). The pH values were found to be slightly acidic. The

level of elements (Phosphorus, calcium, magnesium, potassium, sodium etc.) in the treated soil where all significantly different because of the level of contamination in the treatment is 2%, 4% and 8%.

Table 1 Physicochemical Properties of Experimental Soil.

Parameters	Unit	T0	T1	T2	T3	T4
pH		4.40	4.80	5.02	4.82	4.76
Total Nitrogen	%	0.260	0.218	0.226	0.203	0.154
Clay	%	14.0	11.4	11.4	11.4	13.4
Silt	%	21.4	13.4	13.4	15.4	11.4
Sand	%	64.6	75.2	75.2	73.2	75.2
ECEC	%	22.126	25.197	22.810	23.822	22.005
Acidity	mol/kg	0.32	0.24	0.40	0.24	0.52
P	mg/g	42.711	58.606	53.479	57.581	39.378
Ca	mg/g	20.362	23.481	20.983	22.186	20.025
Mg	mg/g	0.662	1.208	1.176	1.150	1.141
K	mg/g	0.091	0.134	0.125	0.123	0.159
Na	mg/g	0.692	0.134	0.125	0.123	0.159
Al	mg/g	0.00	0.00	0.00	0.00	0.00
Oc	mg/g	2.505	2.105	2.178	1.960	1.488

From table 2 below, T_2(2%) had the highest level of total heterotrophic bacteria (THB) with a value of 7.7×10^7, followed by T_4(8%) (5.3×10^6), T_0(Control) (3.6×10^6) and T_1(Control + amendment) (3.5×10^6). T_3(4%) had the lowest total heterotrophic bacteria (THB), with a value of 3.2×10^6. For the total fungi (TF) content in the soil, T_0(Control) had the highest value 5.5×10^4, followed by T_3(4%)(3.5×10^4), T_1(Control + amendment) (5.5×10^4), T_4(8%)(2.5×10^4). The lowest amount of total fungi in the soil was seen in T_2(2%) with a value of (2.1×10^4). In hydrocarbon utilizing bacteria (HUB) T_1(Control + amendment) had the highest value 7.5×10^5, followed by T_0(Control)(7.1×10^5), T_3(6%) (6.6×10^5) and T_2(2%) (5.3×10^5). T_4(8%) recorded the lowest level of hydrocarbon utilizing bacteria with a value of 2.7×10^5. In the hydrocarbon utilizing fungi (HUF) T_1(Control + amendment) had the highest amount of hydrocarbon utilizing fungi with a value of 5×10^4, followed by T_3(4%) (1.3×10^4) and T_4(8%) (1.3×10^4) .This had the same amount of hydrocarbon utilizing fungi in the soil. T_2(2%) recorded the lowest level of the hydrocarbon utilizing fungi. The various treatments where all significantly different after going through these parameters, because of the level of contamination in them; 2%, 4% and 8% petrol contamination.

Table 2 Microbial analysis of Experimental Soil at various Treatment Levels

S/N	Samples	THB (CFU g^{-1})	TF (CFU g^{1})	HUB (CFU g^{-1})	HUF (CFU g^{-1})
1.	T_0	3.6×10^6	5.5×10^4	7.1×10^5	1.2×10^5
2.	T_1	3.5×10^6	3.3×10^4	7.5×10^5	5×10^4
3.	T_2	7.7×10^7	2.1×10^4	5.3×10^5	1.1×10^4
4.	T_3	3.2×10^6	3.5×10^4	6.6×10^5	1.3×10^4
5.	T_4	5.3×10^6	2.5×10^4	2.7×10^5	1.3×10^4

Figure 1 shows the rate of heavy metals in the plant. From the figure it was observed that T0 which is the control had no trace of Nickel in it, T1(Control + amendment) also did not have any trace of nickel, while other treatments had little amount of nickel at the same level. On the other hand, zinc was found in all the treatment with T3(4%) and T4(8%) having the highest amount of zinc, followed by T2(2%) and T1(Control + amendment). The lowest amount of zinc was observed in T0 which is the control with 0% pollutant.

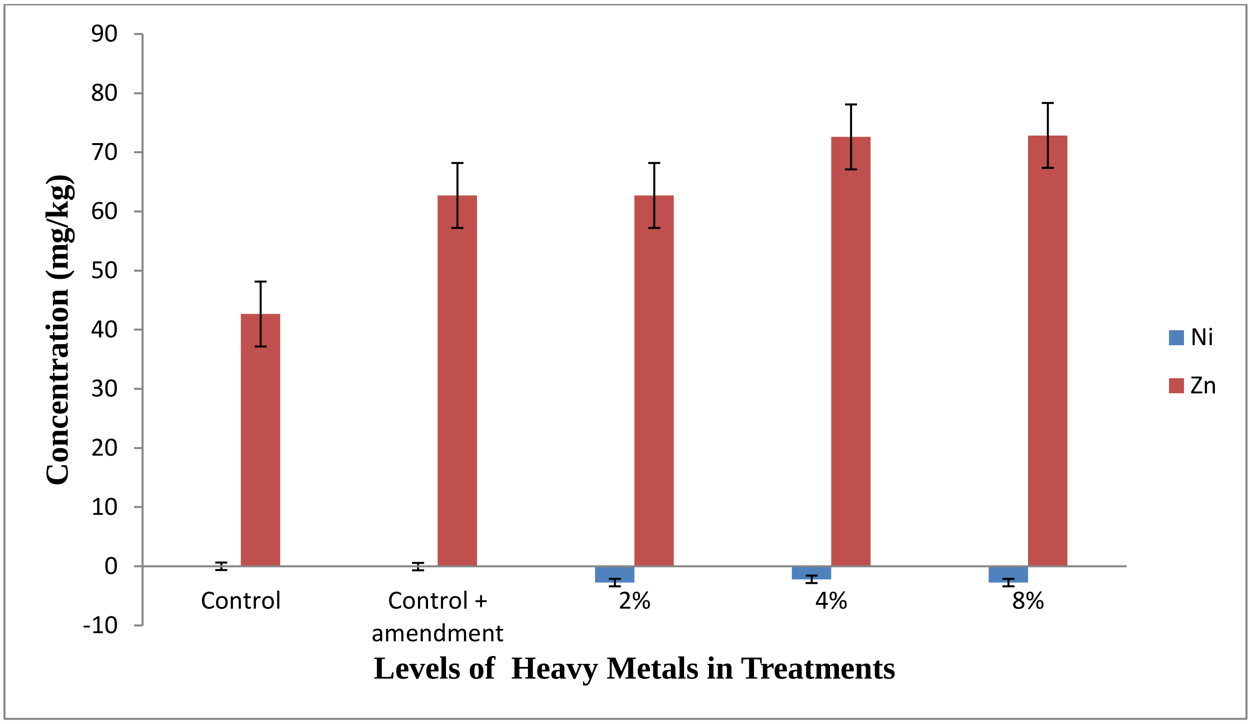

Figure 1 Heavy Metal Analysis showing the rate of Nickel and Zinc at various treatment levels.

Figure 2 shows the plant height at 10 weeks after planting. Control had the highest plant height, followed by 2% petrol amended soil. The lowest plant height was recorded in 8% petrol contaminated soil, while others had lesser growth performance of plant height in this order 4% > control + amendment > 8%.

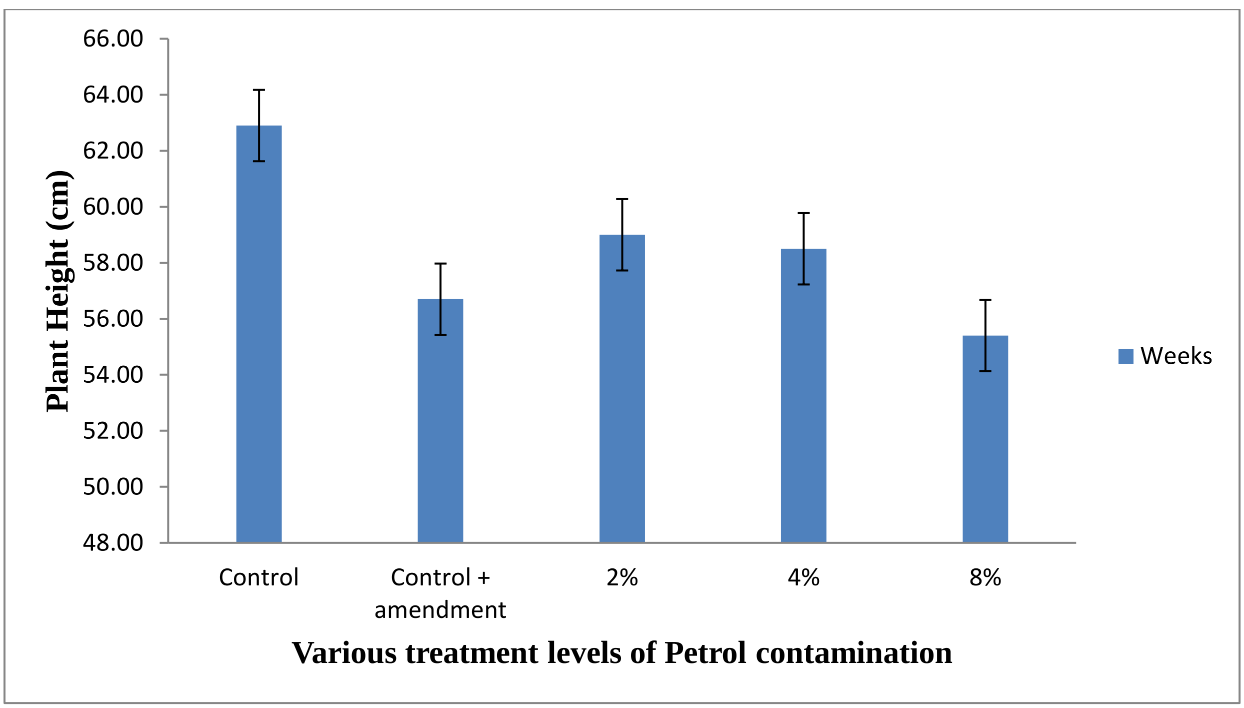

Figure 2 Mean Plant Height of *Vigna radiata* at 10 weeks after planting

Figure 3 shows the number of leaves at 10 weeks, after planting. Control + amendment had the highest number of leaves, followed by 4% petrol amended soil. The least number of leaves was obtained in the control soil, while others had lesser number of leaves in this order 8% > 2% > control.

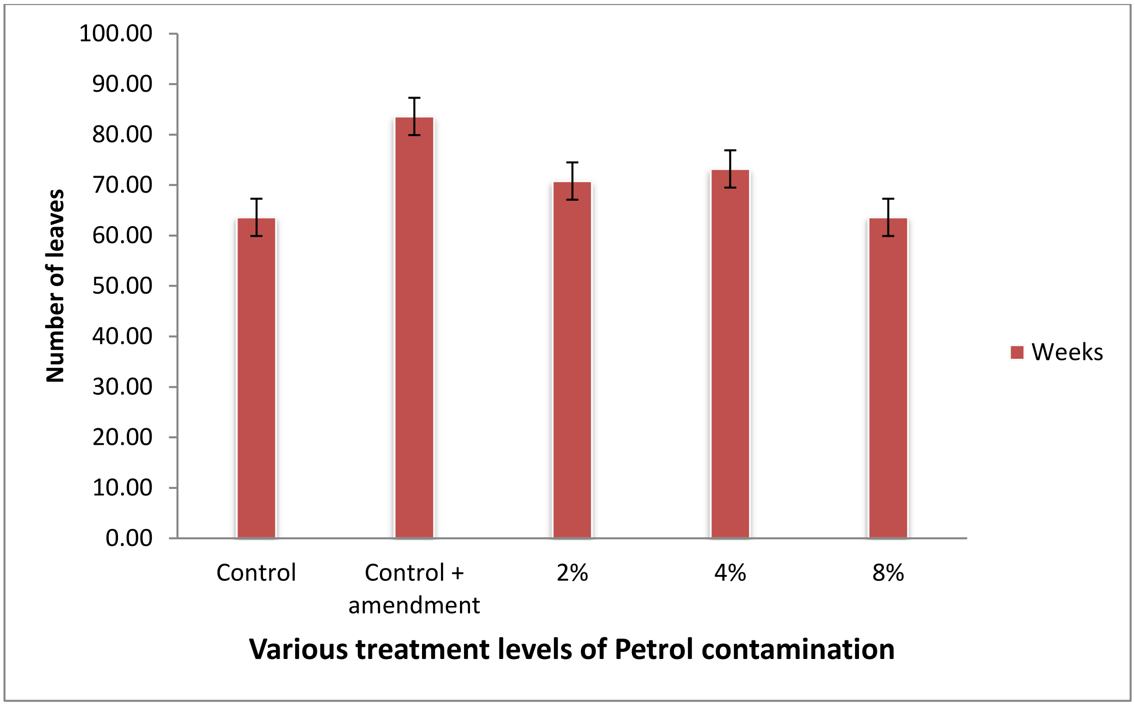

Figure 3 Mean number of leaves of *Vigna radiata* at 10 weeks after planting

In petrol contaminated soil, the fresh weight and dry weight of Vigna radiata decreased with increase in the level of petrol contamination as showed in figure 4. Control had the highest fresh weight, followed by control + amendment and 2% petrol amended soil had the same level of contamination. 4% and 8% petrol amended soil recorded the lowest value for fresh weight. In dry weight 2% and 8% had the highest level of petrol contamination soil amended with *Centrosema pubescens*. The lowest dry weight of the plant was recorded in control + amendment, followed by the control and 4% petrol contaminated soil. Analysis of variance (ANOVA) shows that there were significant difference (P=0.05) among the treatments in fresh weight.

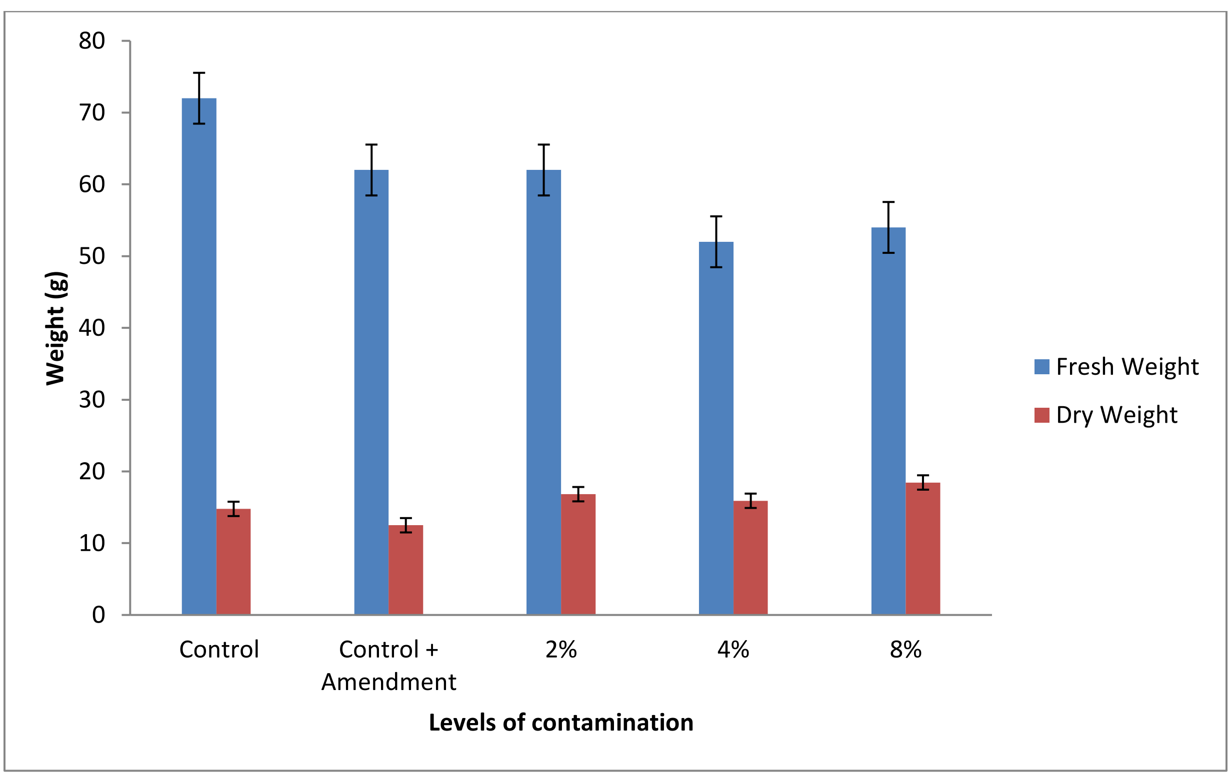

Figure 4 Mean Fresh weight and Dry weight (g) of Vigna radiata

The study showed that the presence of petrol (PMS) had significant and obvious effects on the growth and development of *Vigna radiata* in terms of plant height, number of leaves, fresh weight and dry weight. It was ascertained that the plants had poor growth because of the increased in the amount of petrol (2%, 4% and 8%). The total height of the control of *Vigna radiata* (73.00cm) was significantly greater than the plants grown in soil polluted with 100ml, 200ml and 400ml of petrol. The reduction in growth and biomass performance of the plant could be due to unfavourable soil conditions mainly due to insufficient aerations, following a decrease in the air filled pore spaces (Atuanya, 1987), effects on soil microbes (Ekundayo and Benka – Coker, 1995) and a disruption in the soil water – plant interrelationship (Agbogidi, 2011). The reduction in growth and biomass performance of *Vigna radiate* were due to high level of petrol, it could also be attributed to deficiency in availability of nutrients needed to maintain physiological processes involved in plant growth, occasioned by nutrient stress due to influence of petrol.

4. CONCLUSION

This study was designed to check and amend the negative effects of petrol contamination using organic supplement to remediate the contaminated soil. It took the crop 10 weeks for its seed to germinate, grow and produce mature pods. On the basis of its agronomic characteristics, the crop can be grown many times in a year. This can contribute to boost food production in the country. Therefore; Petrol inhibited the growth of *Vigna radiata*. Petrol had deleterious effects on the growth, yield and even the soil where the crop was planted. The rate of growth and productivity decreased with the increase in the concentration of the petrol in the soil i.e. 2%, 4% and 8% v/w.

Therefore, the use of organic supplement to remediate the polluted soil would enhance and improve *Vigna radiata* cultivated in such environment.

Recommendation

This study will be beneficial to the environmentalist in view of the ameliorative potentials of *Centrosema pubescens* in petrol contaminated soil in relation to the growth of *Vigna radiata*. Thus, *Centrosema pubescens* is recommended for use as amelioration supplement in petrol contaminated soil.

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Conflict of Interest: The authors declare that there are no conflicts of interests.

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